

Determination of Caffeine, the Active Ingredient in Different Coffee Drinks and its Characterization by FTIR/ATR and TGA/DTA

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Abstract— In this article a special attention has been given to the use of FTIR/ATR, a unique accessory of Fourier Transform Infra-red Spectroscopy. This technique uses attenuated total reflectance accessory (ATR) as a sampling system for determination and quantification of caffeine content in coffee samples at the wavenumber (cm^{-1}) of the pure caffeine spectrum selected between ($1600\text{--}1700\text{ cm}^{-1}$) where any possible traces of chloroform does not show any absorption [1]. Attenuated Total Reflectance accessory represented a simple technique that requires a very little sample preparation [2]. In order to reach a meaningful approach of sample characterization, Thermal Gravimetric Analysis (TGA) has been included in the research to identify the thermal properties of coffee samples selected for the study [3]. The targeted coffee samples represented the coffee samples consumed, particularly: Arabian coffee blend home-made, roasted coffee blend home-made, instant coffee bought from the market and pure caffeine analytical reagent bought from Sigma Chemicals.

Index Terms— FTIR/ATR, TGA/DTA, Arabian Coffee blend, roasted coffee blend, Instant Coffee, caffeine.

I. INTRODUCTION

Caffeine or 1,3,7-trimethylxanthine exists as white powdered alkaloid with an intensely bitter taste. Caffeine acts as stimulant for the central nervous system (CNS) having an instantaneous effect on restoring arousal in both humans and experimental animals. Caffeine formula is $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$. Molecular weight 194.19 g/mol . Melting point 237°C . Boiling point 178°C (sublimation temperature). Density 1.05 g/cm^3 . pK_a 10.4 at 40°C . Caffeine, a well-known name, with some possible impact on human health, for that reason, a lot of interest has been directed towards more investigation on it as other components a represent in it which have controversial health effects [4–9]. Caffeine is a methylamine whose Primary biological effect is the antagonism of adenosine receptor [4]. The instant physiological consequences of caffeine intake include, increased blood pressure, increased serum fatty acids, increase in plasma catecholamine levels, increase in duration and increased gastric acid secretion. Caffeine has pharmacological uses as a cardiac and respiratory stimulant and diuretic in small doses which is present in an average cup of coffee ($100\text{--}200\text{ mg}$).

High concentration of caffeine can lead to addiction, ulcer, breast cancer, coronary diseases, myocardial infarction [4–5] and death if the lethal dose is reached around 10 to 15 g.

Manuscript received .

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However moderate use of coffee has little effects if it is not used in association with smoking as it is a habit that happens so often [4,5,6,7,8]. The article sheds light on the samples used in the research. The coffee samples were selected to represent the mostly used qualities, namely: 1-Arabian coffee blend which consists of green coffee partially roasted to a yellow color then powdered with little cardamom, a spice mostly used in Arabian peninsula as coffee additive. Also people in Arabian societies eat dates while drinking coffee. Cardamom spice is known for treating digestive problems including heartburn, intestinal spasm, intestinal gas, constipation, liver, gallbladder complaints, common cold, loss of appetite. Some people use this spice as a stimulant for urinary problems (ref- WebMD) 2/Roasted coffee blend home prepared by roasting coffee beans completely to a black product then powdered with dry ginger. Ginger is known to have curative benefits. Some of the curatives have been discovered by researchers in the treatments of cancer to migraines. A study was conducted at the university of Michigan Comprehensive Cancer Center found that ginger induces cell death in ovarian cancer cells to which it was applied. Cinnamon is some time added with powdered ginger or cloves to roasted coffee as a spice mixture additive. Cinnamon is known to have an anti-ulcer curative effect on ulcers possibly caused by the acidity of organic acids present in coffee. Cloves oil, an active ingredient of cloves is known to treat many health disorders including headache, indigestion, cough, blood impurities. Cloves is mostly used in dental care and as an antiseptic, antiviral cure. The spices additives to roasted coffee and Arabian coffee have been used for many centuries in Arabian, African and Asian countries [9] 3/ Instant coffee of commercial origin has been bought from the market for comparison with the other coffee samples with no details on the preparation recipe. 4/ Pure Caffeine has been included as a reference sample.

II. EXPERIMENTAL

A. Apparatus

FTIR Nicolet 6700 equipped with Attenuated Total Reflectance accessory designed for use with a diamond crystal ($200\text{--}30000\text{ cm}^{-1}$) to measure the reflectance of solid and liquid samples. The spectra can be transformed by recording the absorbance as a function of wavelength. The concentrations can be calculated from absorbance measurements using a calibration curve. The interferogram is equipped with KBr beam splitter and DTGS/KBr detector.

Conditions of measurements: wavenumbers $400\text{--}4000\text{ cm}^{-1}$, number of scans 32, resolution 4 cm^{-1} .

III. METHODS

A. Preparation of samples

Solid samples

Arabian coffee blend: partially roasted beans was blended with Cardamom spice in the ratio of (100:1) and stored at room temperature for analysis. Roasted coffee blend: Completely roasted coffee beans was blended with Ginger and Cloves spices in the same ratio and stored for analysis. Instant coffee: roasted coffee of commercial origin. Pure caffeine used as a standard sample. Aqueous extracts of the coffee samples were prepared by boiling (1g) of each in about 70ml of distilled water for ten minutes, cooled, completed to (100ml) in volumetric flasks and filtered through Whatman filter paper No. 1. The extracts represented almost the concentration of coffee consumed in a 100 ml cup of coffee. Caffeine content of each was measured by FTIR/ATR.

B. Standard Caffeine

(1g) Caffeine in 100 ml Chloroform.

Procedures

Preparation of standard caffeine

Standard solutions of caffeine containing 0, 2, 4, 6, 8 and 10 mg in chloroform were pipette separately in 100 mg dry KBr. The standard samples were mixed, crushed and dried under N_2 gas to remove traces of chloroform.

Preparation of coffee samples:

Solid coffee samples finely powdered were used directly for analysis.

FTIR/ATR data collection for the standard samples and coffee samples under investigation were collected after collecting the background spectrum. Solid samples of standard and coffee were layered on the crystal. Each interferogram of caffeine films was rationed against the background interferogram and the result was collected in absorbance.

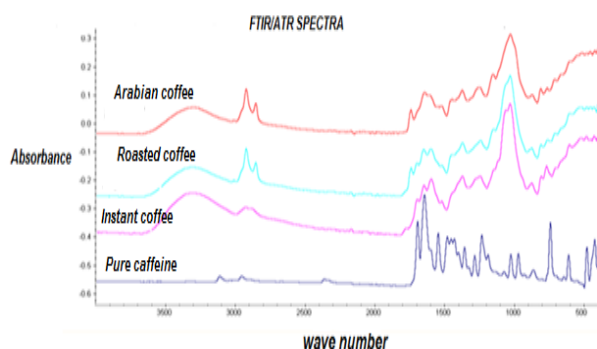
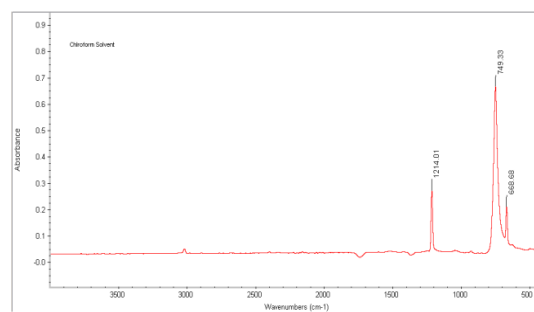


Fig 1a. FTIR/ATR spectra of solid samples of pure caffeine and coffee samples: from top to bottom, Arabian coffee blend, roasted coffee blend, Instant coffee and caffeine standard respectively.

Fig 1 b- below is chloroform spectrum, shows no interfering absorption between $1600-1700\text{ cm}^{-1}$.



Figures 2a, 2b, 2c, 2d below show the spectra of coffee samples and caffeine with their respective wavenumbers.

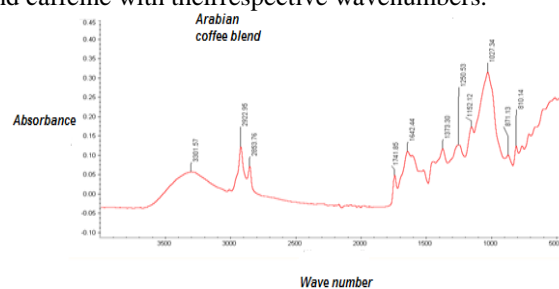
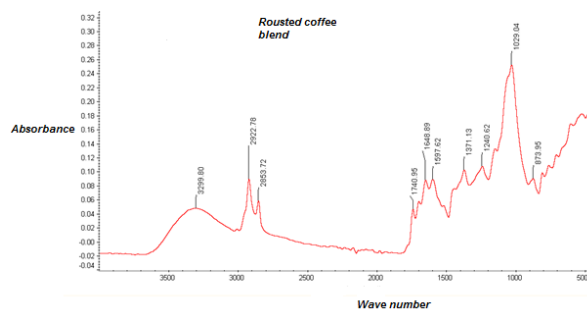


Figure (2a)



Figures (2b)

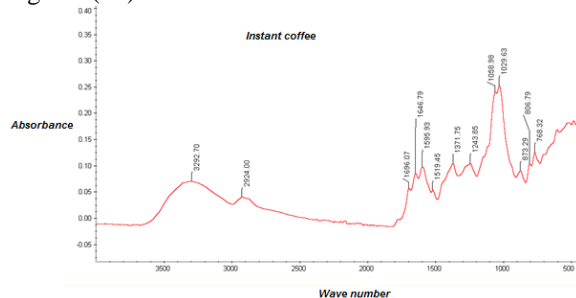


Figure (2c)

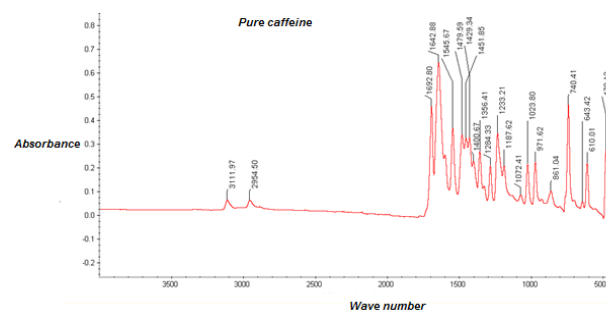
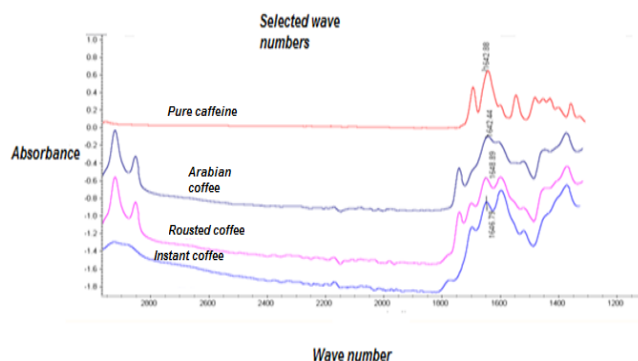


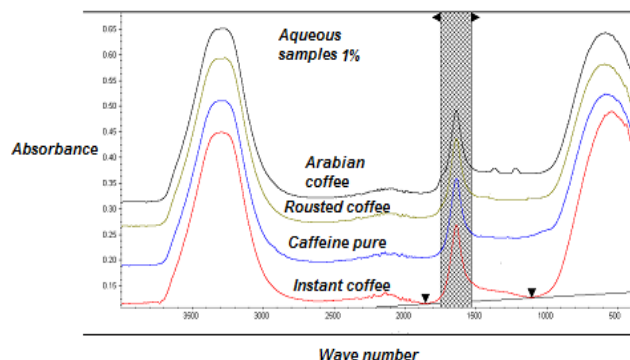
Figure (2d)

The spectra below show the wavenumber selected from pure caffeine as a standard wavenumber for quantification of caffeine content in coffee samples. The slight shift from the wavenumber 1642.44 cm^{-1} of caffeine was due to the preparation process of coffee extracts, e.g. roasting.

procedure and temperature. Arabian coffee wavenumber 1642.44 cm^{-1} . Roasted coffee 1648.69 cm^{-1} . Instant coffee 1646.79 cm^{-1} .



Aqueous samples (1%) in distilled water for coffee samples and caffeine were introduced in the text to represent approximately the coffee concentration consumed in (100ml) extract. The overlay of spectra below, figure 3, shows 1% aqueous extracts of Arabian coffee, roasted coffee, pure caffeine and instant coffee.



The measured pH of extracts were: 4.5, 5.0, 5.0 and 5.1 for Arabian coffee, roasted coffee, caffeine and instant coffee respectively.

TGA experiments were run on coffee samples under investigation and pure caffeine to study the thermal properties of each, using Mettler Toledo TGA-DSC 1. Weights of crushed, dry samples under 10mg were weighed in 70 ml alumina crucibles and placed on the precision balance. The samples were heated in a constant heating rate mode from ambient to 700°C at $10^\circ\text{C}/\text{min}$. The acquired thermograms demonstrate the effect of heat flow on samples.

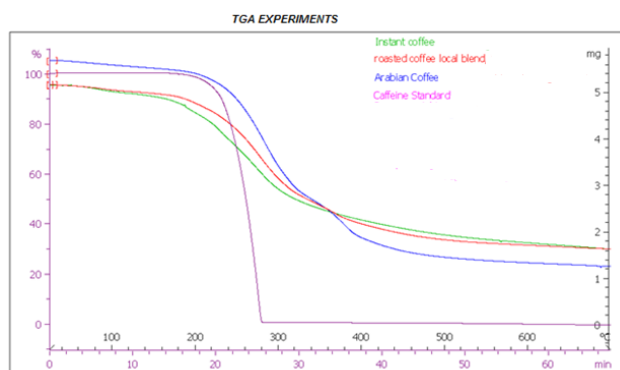


Figure-4- above demonstrates the thermograms of the samples with percentages loss of masses caused by heat flow through coffee samples.

A set of TGA experiments were also performed to show the loss of masses due to volatiles from ambient to 200°C at the same rate ($10^\circ\text{C}/\text{min}$).

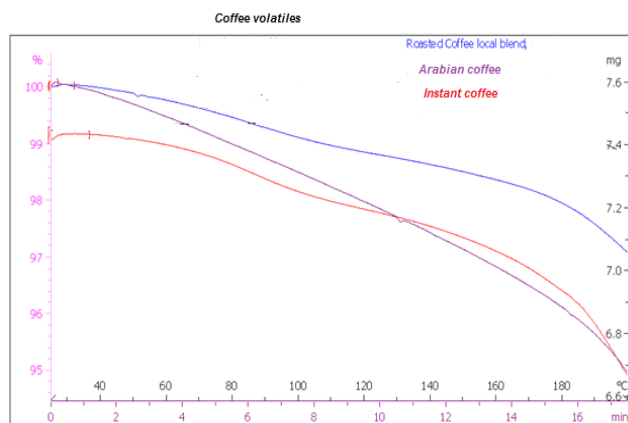


Fig 5 above : shows in the thermograms the percentage loss in mass in each sample after heating from ambient temperature to 200°C .

TGA /DTA

TGA measures changes caused by mass loss. Differential Thermal Analysis (DTA), first derivative of TGA have been introduced in the text. The material under study and an inert reference are made to undergo identical thermal behavior. Any temperature difference between sample and reference is plotted against temperature. DTA phenomena causing changes in heat/temperature. Physical adsorption (exothermic), desorption (endothermic), vaporization (endothermic, sublimation) [10] Combining the two techniques (TGA/DTA) gives a comprehensive behavior of coffee samples and caffeine and characterize thermal stability and sample purity [11]. The thermograms in figures 6, a, b, c, d. show a combination of the two techniques.

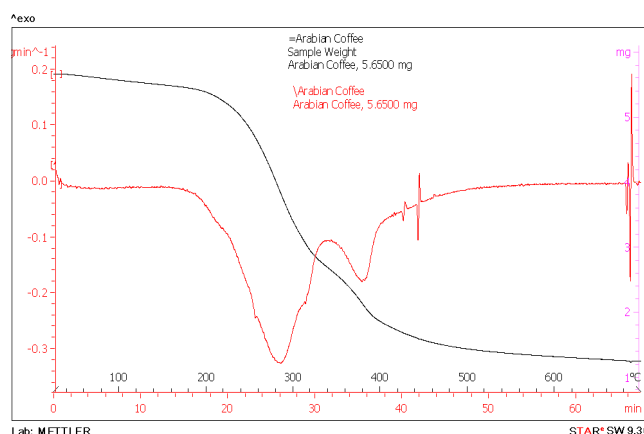


Figure (6 a) shows TGA/DTA thermogram of Arabian coffee.

Sample	Absorbance	Caffeine%
Arabian coffee	0.26	5.050
Roasted coffee	0.38	7.053
Instant coffee	0.44	8.636

Figure 6 b, shows TGA/DTA thermogram of instant coffee.

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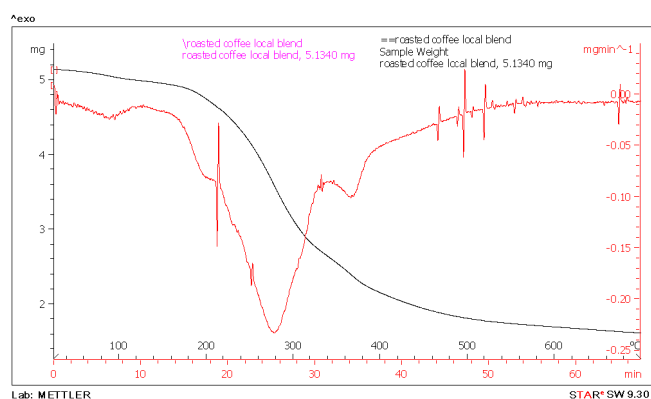


Fig 6c shows TGA/DTA thermogram of roasted coffee.

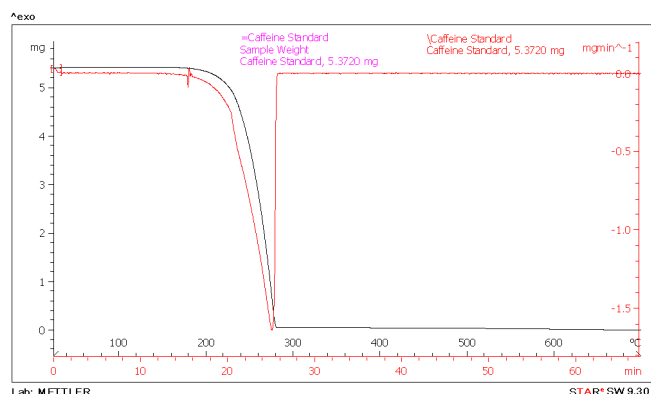


Figure.6d shows TGA/DTA thermogram of pure caffeine.

Results and discussion

A standard curve of absorbance vs % caffeine in KBr is shown in Fig.7. Table 1 shows % caffeine in dry coffee samples.

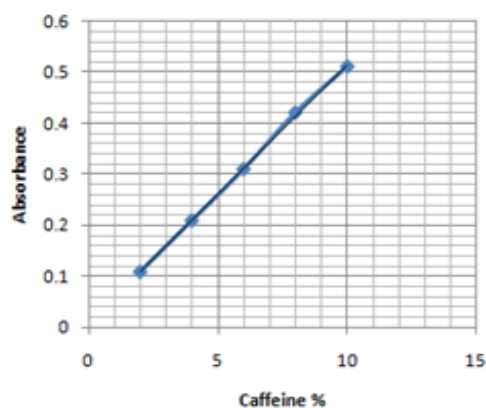


Figure (7)

A standard curve of aqueous caffeine was prepared by diluting 1% caffeine a hundred times in distilled water and extracting the aqueous caffeine contents of 20, 40, 60, 80, 100 μ l in 0.5 ml chloroform then diluting the extracts in 100 mg KBr, grinding the mixture and evaporating chloroform before taking the FTIR/ATR spectra. A standard curve of absorbance vs ppm caffeine was drawn for calculating the caffeine contents in aqueous coffee samples. Volumes of 100 μ l from aqueous coffee samples were each extracted in 0.5 ml chloroform, then diluted in 100 mg KBr for FTIR/ATR analysis. The standard concentrations of caffeine were 20, 40, 60, 80 and 100 ppm respectively. The concentrations of

caffeine in coffee samples in ppm was obtained by multiplying the results from the curve by a factor of ten.

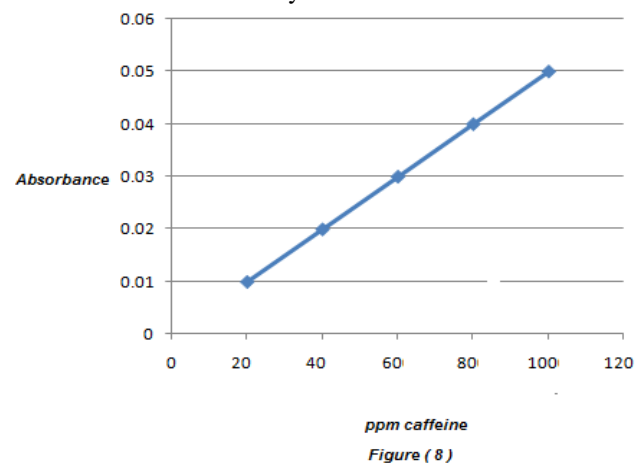


Figure (8)

Figure 8 shows the absorbance of standard aqueous caffeine concentrations in ppm.

Table 2 shows the concentrations in ppm of 1% aqueous coffee samples analyzed.

Coffee sample	Absorbance	Concentration of caffeine in ppm
Arabian coffee	0.016	32.3
Roasted coffee	0.022	45.2
Instant coffee	0.340	68.3

The quantification of coffee samples analyzed indicated that the average mass of caffeine content consumed in a 100 ml coffee cup, was around 32.3, 45.2 and 68.3 milligrams for Arabian coffee, roasted coffee and instant coffee respectively.

Table 3 shows loss in mass of coffee samples because of heat flow in TGA thermograms.

Coffee sample	% loss in mass at 200°C	% loss in mass at 700°C
Instant coffee	8.63	68.62
Roasted coffee	6.17	68.70
Arabian coffee	5.05	78.09

The rate in mass loss at 200 °C is mostly pronounced and closely related to caffeine because of sublimation. The mass loss at 200 °C was high in instant coffee and low in Arabian coffee because of roasting temperature and time. In Arabian coffee moderate roasting temperature reserved caffeine in the beans and permitted slow release of it to the surrounding compared with other samples. Mass loss at 700 °C gave the highest percentage to Arabian coffee. The DTA study of coffee samples illustrated on the thermograms indicated that the caffeine content of Arabian coffee sample (Fig. 6 a), gave a clear thermal group that agreed with that of standard caffeine. The other thermal group that followed was related to spice additives. The same result was shown in roasted coffee. The result of instant coffee DTA illustrated the presence of a distorted caffeine peak with minor peaks in the surrounding which belonged to impurities.

IV. CONCLUSION

Fourier transform infrared spectroscopy has proved to be a good technique for quantifying a wide range of materials especially pharmaceutical products when it is used in combination with Attenuated Total Reflectance Accessory. TGA/DTA a combined technique in thermal analysis measures the physical changes and chemical changes that occur in characterizing the samples to show thermal stability and material purity. The purity is compared with pure references. The aim of the research has been focused on coffee samples containing spices to know their effect on caffeine. The results showed the absence of any chemical effect on the caffeine content of coffee samples as exhibited in the FTIR/ATR spectra. The purity of caffeine in Arabian coffee and roasted was maintained in the thermogram whereas the Instant coffee showed signs of impurity in the thermogram. Finally, the presence of spices in Arabian coffee and roasted home made coffee offers curative benefits and preventive medication as presented in the introduction which is good for the health of coffee consumers.

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